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To: .Dr. C. K. Ellis Date: November 15, 1988

From: .R. D. Kinser and A. H. Warfield

Subject: .TSNA Priority Program Operational Plans: Revised after Third Quarter 1988 Planning Meeting

OBJECTIVE: To design a product by 1991 with MS TSNA (TSNA/mg TPM) delivery reduced 90% relative to the TPM corrected TSNA delivery of a 1987 full-flavored, blended cigarette.

STATUS AND BACKGROUND

Previous studies have indicated that mainstream (MS) TSNA arise from pyrosynthesis during smoking and transfer of filler (endogenous) TSNA into the smoke stream, and that the total delivery is also affected by some TSNA decomposition during the smoking process. Increased understanding of the formation of TSNA during curing has been obtained from two extensive curing studies, but current work aimed at reduction of TSNA transfer (distillation) is focused on selective removal of TSNA from cured filler. A continuous process whereby TSNA and alkaloids are extracted from filler and then removed from the extraction solvent by an ion exchange resin has been developed. The possibility of reducing endogenous TSNA by biochemical alteration of tobacco, resulting in lowered biogenesis of alkaloids, is being examined.

Research on the inhibition of TSNA pyrosynthesis has indicated that the amine precursors of NNN and NAT are the secondary amines nornicotine and anatabine. The amine precursor of NNK has not been identified, but our research indicates that nicotine is not the primary amine precursor of MS NNK. Disproportionately high levels of MS NNK from base webs and base webs extracted with organic solvents suggest the possibility of a "bound" form of an amine, such as "unextracted nicotine", may be the NNK precursor. Experiments to investigate this hypothesis have been initiated. Although addition of a primary amine to tobacco does not appear to be a viable means of reducing MS TSNA (NNN, NAT, and NAB are actually significantly increased), the small reduction in MS NNK following such addition will be examined further. Model studies have indicated that antioxidants accelerate TSNA thermal decomposition, that ascorbyl palmitate is more effective than propyl dihydroxyhydrocinnamate, and that NNN and NAT are more readily decomposed than NNK. TSNA pyrosynthesis does occur during the smoking of oriental (Or) tobacco when nitrate and alkaloid levels are increased to approximate the levels of these probable precursors in burley tobacco. However, the smoke from an RL made from burley and oriental CELs applied to Bu base web delivers less MS TSNA than the smoke from an RL containing the same amount of BuCEL and no OrCEL. Addition of ascorbic acid to this RL further decreases TSNA delivery, suggesting that the OrCEL affects pyro-synthesis while the antioxidant causes decomposition of endogenous TSNA. Studies to

further evaluate the "oriental inhibitor" are planned. Cigarette circumference appears to have no effect on MS TSNA delivery on a gram filler consumed basis. Paper porosity and packing density will also be evaluated.

STRATEGIES

These plans assume that Philip Morris chooses not to exert significant influence on tobacco cultivation, and therefore concentrate on tobacco treatment methods for decreasing TSNA delivery by distillation and methods which inhibit TSNA pyrosynthesis. Control of TSNA formation requires a greater understanding of those processes than currently possessed by us or described in the scientific literature, and the plans formulated to address the various strategies include defining the mechanisms of TSNA pyrosynthesis and of TSNA transfer into mainstream smoke. This basic research also includes a strategy designed to evaluate the possibility of TSNA reduction by biochemical alterations to the tobacco plant. The plans also include examination of various a priori methods for TSNA reduction. The target date represents our best prediction for a development model without flavor optimization meeting the 90% reduction (relative to a 1987 full-flavored, blended cigarette) goal using technologies and knowledge not available at this time. The priority assigned to each strategy is indicated by the number preceding the strategy. Tactics will be designed for achievement of Strategy 9 as more information about various additives becomes available.

REDUCTION OF MS TSNA BY INHIBITING THE PYROSYNTHESIS OF TSNA

1. Reduce the levels of pyrosynthesized MS TSNA by incorporation into the cigarette design those aspects of oriental filler which result in an absence of significant TSNA pyrosynthesis from oriental tobacco.

2. Reduce the levels of pyrosynthesized MS TSNA by removal of the amine precursor(s), or decreasing the reactivity to nitrosation of the amine precursor(s).

3. Reduce the levels of pyrosynthesized MS TSNA by removing nitrosating agent(s) or precursor(s) of nitrosating agent(s), or blocking reaction pathways which form nitrosating agent(s) or which yield TSNA from the nitrosating agents.

REDUCTION OF MS TSNA BY REDUCING ENDOGENOUS TSNA IN FILLER

4. Reduce MS TSNA by selective removal of TSNA from filler.

5. Reduce MS TSNA by decreasing endogenous TSNA by biochemical alteration(s) to tobacco.

REDUCTION OF MS TSNA BY ENHANCING DECOMPOSITION OF TSNA

6. Evaluate the enhancement of TSNA decomposition during smoking as a method for reducing TSNA delivery.

REDUCTION OF MS TSNA BY ALTERING PHYSICAL/CHEMICAL PARAMETERS OF CIGARETTES

7. Reduce the levels of pyrosynthesized MS TSNA by alterations in cigarette construction parameters.

REDUCTION OF MS TSNA BY ALTERING PHYSICAL/CHEMICAL PARAMETERS OF CIGARETTES (continued)

8. Reduce the levels of pyrosynthesized MS TSNA by manipulation of filler salt content.

9. Reduce the levels of pyrosynthesized MS TSNA by manipulation of casings typically used in cigarettes but missing from the reference cigarette.

TACTICS AND TIMETABLE

Outlined below are detailed plans for the remainder of 1988 and an overview of work planned for 1989 and 1990. Timeframes given are best estimates possible at this time and represent updates based on review of this plan at the end of the Third Quarter, 1988. No attempt has been made to allow time for possible analytical or instrumental problems; any schedule revisions needed due to these causes will be made on a quarterly basis. Included in the present revision are requests received to date from other priority programs for TSNA analyses.

FOURTH QUARTER, 1988

Oriental Inhibitor Strategy

Determine MS smoke TSNA of selected solvent-extracted Bu fillers and evaluate as potential substrate for Or tobacco component studies	Oct. 21
Prepare enough Bu - TSNA - nicotine filler (DBC Bu depleted of TSNA and nicotine but containing minor alkaloids) for use in subsequent studies	Nov. 1
Initiate organic solvent extraction of Or filler to evaluate extract as inhibitor of TSNA formation	Nov. 7
Determining effect of "local" cultivation on chemistry of oriental tobacco	Dec. 15
Oriental Model Study: Effect of OrCEL fractions on NNN formation from nornicotine and nitrite	Dec. 31
Overspray OrCEL and fractions on Bu - TSNA - nicotine filler Evaluate for inhibition of pyrosynthetic activity	Dec. 31

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Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
-Size nicotine bitartrate crystals and submit for preparation of microencapsulated nicotine	Nov. 30
-Prepare and evaluate nicotine release properties of a nicotine-amino acid [t-butyl 2-(3-pyridinyl)-1-pyrrolidinylacetate]	Dec. 31
-Extract green burley tobacco to remove nicotine, cure; prepare cigarettes, smoke, and analyze TSNA	Dec. 31
Replicate Hoffmann ^{14}C-nicotine addition:	
-Complete method development and assess purity of ^{14}C-nicotine	Dec. 31

Nitrosating Agent Strategy

Evaluate results of FTIR-EGA experiments carried out under oxidative conditions	Oct. 31
Add sugar and/or antioxidant to BuCEL on BuBW to test hypothesis that sugar/ammonia reaction produces scavengers of nitrosating agents active during TSNA pyrosynthesis	Oct. 31
Develop methodology and test flash heating of TPM to determine if TSNA precursors for pyrosynthetic type reactions are present	Dec. 31

Removal of Endogenous TSNA Strategy

Begin final stages of development of laboratory process for preparation of ExBu filler (TSNA, nicotine and minor alkaloid-depleted) from DBC burley:	
-Extract larger DBC burley filler sample	Nov. 1
-Prepare cigarettes from ExBu filler	Nov. 8
-Determine filler and MS smoke TSNA from ExBu filler	Dec. 15
-Analyze extract generated in above process for TSNA and alkaloids	Nov. 30
Project Art Support:	ongoing
-Alkaloid analyses of samples from Supercritical Processing	Nov. 14
Sepracor Support	ongoing

Biochemical Alterations to Tobacco Strategy

Classical purification methods	
-Complete method development	Nov. 7
-Prepare purified material in bulk (25 X enrichment, >60% recovery)	ongoing

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Biochemical Alterations to Tobacco Strategy (continued)

Immunological approach	
-Preparation of antibody from partially purified PMT prep	if necessary
Affinity chromatography	
-Continue development and select best system	Nov. 7
Electrophoretic methods	
-Complete development of a continuous flow electrophoresis system	Nov. 7
-Evaluate and apply 2-D separations to purification scheme	Begin Oct. 14
-Initial results from PDI (lab performing 2-D work)	Nov. 18
Complete development of photoaffinity labelling with ^{3}H-SAM	Oct. 28
Apply complete purification scheme to fresh extract	Begin Nov. 30

TSNA Decomposition Strategy

Continue to study effects of antioxidants on decomposition of nitrosamines in order to elucidate their possible mode of action:	
-Complete PrDHHC slow heating study	Oct. 31

Alteration of Physical/Chemical Parameters Strategy

Alteration of cigarette construction parameters:	
-Evaluate effect of paper porosity and packing density	as time permits
Manipulation of filler salt content to reduce levels of pyrosynthesized TSNA:	
-Obtain smoke TSNA of Ca added CSBW's	as time permits

Analytical Methods Development

Develop simultaneous VNA/TSNA GC/TEA analysis	as time permits
Develop SPE method for smoke TSNA workup	as time permits
Develop GC method for nicotine/minor alkaloid analysis	Oct. 31

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1989

Oriental Inhibitor Strategy

If Or solvent extract is active as TSNA pyrosynthesis inhibitor:	
Fractionate Or solvent extract	Jan. 31
Overspray Or extract and fractions on ExBu/ALK filler	Feb. 15
Evaluate above for inhibition of pyrosynthetic activity	Feb. 28
Optimize levels of active fraction(s) from CEL and/or extract	Mar. 15
Identify active component(s)	2nd-3rd Q
Add Or inhibitor + antioxidant to DBC Bu, RL, BW	4th Q
Add Or salt profile to BuCEL on BuBW to test hypothesis that Or salts contribute to the observed Or inhibitor effect	Feb. 15
Effect of pH of oriental	as time permits

Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
Evaluate water-insoluble protein from tobacco	Mar. 31
Replicate Hoffmann ^{14}C-nicotine addition:	
-Prepare labelled cigarettes, perform preliminary smokings, and develop collection methods	Mar. 31
-Perform experiment and obtain analytical results	2nd Q
Investigate additional means of removing bound nicotine from filler	Feb. 28
Investigate proximate NNK precursor:	
Develop derivatization/HPLC method for secondary amines	Mar. 31
Apply above method to filler and smoke	2nd Q

Nitrosating Agent Strategy

Carry out selected reactions of sugars with ammonia and evaluate reaction products as scavengers of nitrosating agents during smoking	Mar. 31
Initiate studies of role of NO in nitrosation	2nd Q
Completion of studies from nitrosation proposal	3rd Q

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Removal of Endogenous TSNA Strategy

Finalize laboratory process for preparation of ExBu filler (TSNA, nicotine and minor alkaloid-depleted) from DBC burley:	
Evaluate cigarettes made from ExBu filler	
-Analytical data	Feb. 28
-Small scale subjectives	Mar. 31
Apply concentrated extract back to ExBu filler	Feb. 28
-Determine MS smoke TSNA on above filler	Mar. 15
-Analytical data	Mar. 31
-Small scale subjectives on above filler	Mar. 31
Project Art Support	ongoing
Sepracor Support	ongoing

Biochemical Alterations to Tobacco Strategy

Determine appropriate alkaloid biogenesis pathway for next modification; perform requisite enzymological studies to identify enzyme for removal; isolate enzyme	ongoing

TSNA Decomposition Strategy

Continue to study effects of antioxidants on decomposition of nitrosamines in order to elucidate their possible mode of action	ongoing

Alteration of Physical/Chemical Parameters Strategy

Alteration of cigarette construction parameters:	
-Evaluate effect of other construction parameters (paper porosity, packing density, etc.)	as time permits
Manipulation of filler salt content to reduce levels of pyrosynthesized TSNA	as time permits
Effect of trace metal content on MS TSNA delivery	as time permits

1990

Construction and evaluation of models based upon studies to date

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RESOURCE ALLOCATIONS FOR 1988

How are the personnel assigned to this program allocated?

	Professionals	Technicians
Oriental Inhibitor Strategy:	0.80	0.60
Amine Precursor Strategy:	0.65	0.50
Nitrosating Agent Strategy:	0.50	0.30
Extraction of Endogenous TSNA Strategy:	0.90	0.20
Biochemical Alteration of Tobacco Strategy:	6.20	
Decomposition of TSNA Strategy:	0.75	
Cigarette Construction Parameters Strategy:	0.70	0.15
Adjustments to Filler Salt Content Strategy:	0.05	0.05
TOTAL	10.55	1.80

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